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Melatonin and the pineal gland - From basic science to clinical application
Y. Touitou, J. Arendt and P. Pévet, editors.

Rat serum melatonin rise and fall: Influence of morning light

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INTRODUCTION

Illnerova and Vanecek [1-6] adapted rats to various lengths of dark (scotoperiod) with novel light pulses or novel dark extension. The rise and fall of melatonin (MEL) synthesis were each entrainable in dark without an influence of acute inhibition by light gating the surge into dark. Evening light phase-delayed and morning light phase-advanced the MEL rise and fall. Variable and opposite influences on the rise and fall timings and a variable interaction between them depended on scotoperoid length. Usually, the advancing effect of morning light determined the MEL fall. In shorter scotoperiods, evening light had a delaying effect on the MEL rise. Also, particularly in dark lengths above 12 h, morning light could entrain the timing of the MEL rise. In those studies, pineal N-acetyltransferase (NAT) activity was used to index the MEL rhythm in Wistar rats. We have employed two scotoperiods to assess whether similar influences of the evening and morning edges of the light phase can be extended to other rat strains and circulating MEL.

METHODS

Weanling male Sprague-Dawley (SD) and Fischer 344 (F) rats were placed in a cycle with fluorescent ("cool white") lighting (60 foot-candles at cage fronts) for either 14 or 10 h. Middark was at 0100 h. After 8 weeks in one photic regimen, blood and pineals were taken at various times (7-9/group), under dim red safe light for dark phase sampling. Sampling nominally at the transitions between phases was done just before the transition. Additional groups pinealectomized (PX) 8 weeks earlier were sampled (5/group) at midpoints of dark and light. Pineals were assayed for NAT [7] and MEL [8]. Serum MEL was assayed with the Rollag antibody (analytic least detectable 5 pg/ml) after 2 petroleum ether washes of the buffer eluate from chloroform extractions washed with HCl, NaOH, and H_2O [9]. Between-group comparisons were by Bonferroni-corrected t tests. Cyclic data were analyzed by cosinor 24-h periodic regression. In order to compare acrophases by t test using the residual degrees of freedom from the regressions, the coefficients and their standard errors (SE) were used to construct an ellipse giving the SE separately for the angular (acrophase) dimension. Analyses utilized the P7D and P1R programs of the BMDP package on a VAX computer [10].

RESULTS

The nocturnal surge for all MEL variables in both strains and regimens was significant ($p < 0.001$). Figure 1 shows acrophase delay in the longer scotoperiod, significant for pineal and serum MEL in SD and for serum MEL in F. In figure 2, shorter dark was associated with about a 2-h earlier fall of all MEL variables in both strains, consistent with the 2-h earlier morning light despite the 2-h longer evening light. The MEL fall was essentially complete before morning light. F rats showed no regimen-dependent influence on the MEL rise. SD rats had a later rise (significant for serum MEL) in longer dark. PX produced daytime serum values at night, likely representing the noise level of non-specific serum crossreactivity (about 10 pg/ml) in this assay in this species. MEL surge lengthening in longer dark can be inferred from the rise and fall patterns in F (but much less securely in SD) rats.

DISCUSSION

The earlier fall of MEL in shorter dark, accomplished prior to onset of light, indicates entrainment (not gating) of the MEL fall influenced mainly or exclusively by morning light at these scotoperiod lengths in the rat. The MEL rise also appeared not gated, failing to advance

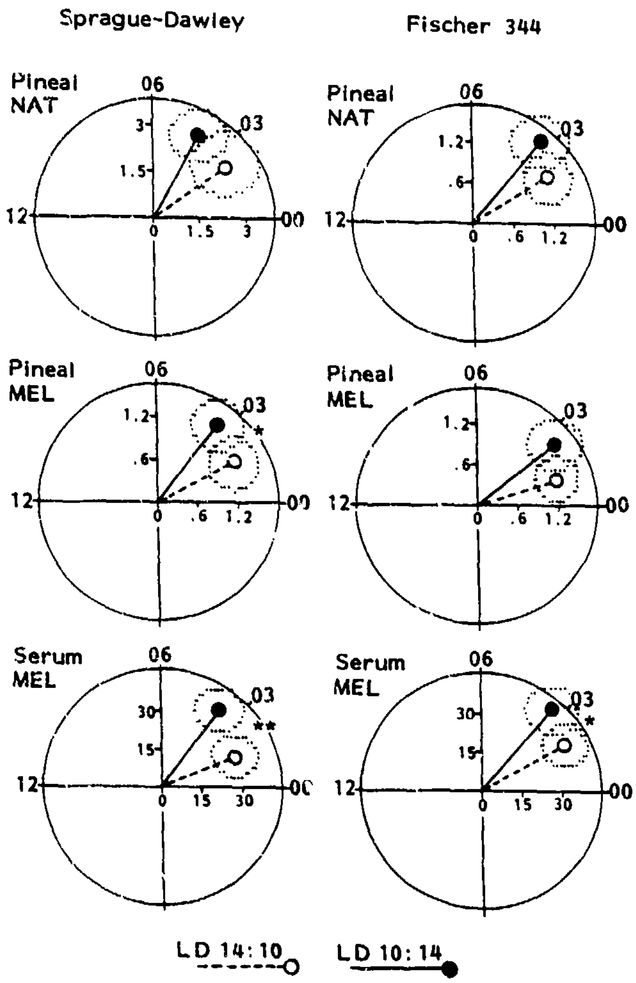

Figure 1. Polar depiction of amplitude (distance from origin) and acrophase (angle from midnight 00 in h marked on the circle) shown as a single point, for melatonin variables. The 95% confidence ellipse of the central point is shown by small dots. *$p < 0.05$, **$p < 0.01$, LD 14:10 vs 10:14 (with respect only to acrophase).

in the longer dark. Lack of difference in MEL rise time between regimens in F rats suggests some separate control of MEL rise and fall. Morning light was more

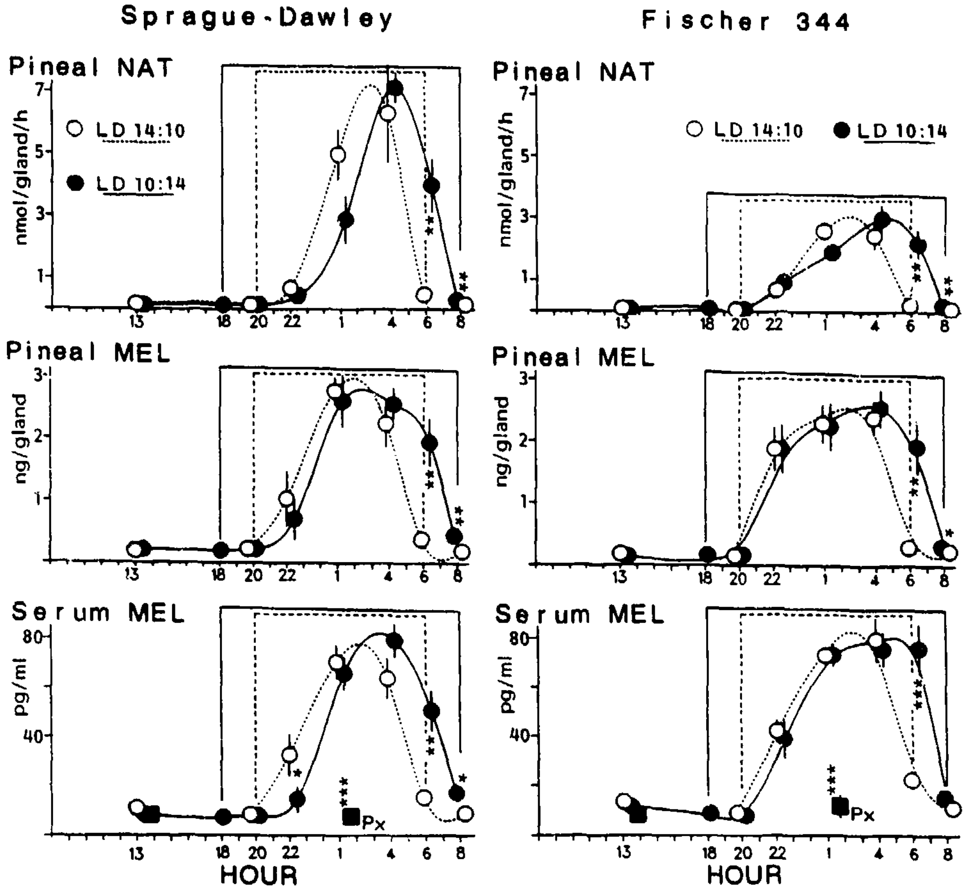

Figure 2. Melatonin variables (mean $\pm$ SE) in two rat strains. The vertical lines connected at their tops define the lengths of the dark phase, different between LD 14:10 (dashed) and 10:14 (continuous). The closed squares (serum MEL) represent Px rats in LD 10:14. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, LD 10:14 vs 14:10, or Px vs the other groups, at the particular time point. The curved lines represent spline fits to the means.

effective than evening light to entrain the MEL rise in SD rats. Overall predominance of morning light effect was also shown by phase-advance of serum MEL acrophase in the short dark regimen in both strains. These data fit previous observations in Wistar rats [1-6] of non-gating two-oscillator entrainment. We now extend this model to two other strains and to circulating MEL itself in the rat. That the human MEL rise may not always follow the altered timing of the end of light when the dark phase is contracted on both sides by bright or natural light [11-14] may result from interference of morning light, as in rats. However, with lack of crisp definition of photic exposure at the edges of the light phase in both control and experimental conditions, typical non-confinement of the entire human MEL surge during darkness, and brighter light required to suppress MEL secretion, it has been difficult to discern human MEL entrainment from gating by evening or morning light. Nevertheless, bright light on two to three waking nights entrained the entire human MEL surge to the new sleep time, as assessed by measurements made without gating in constant conditions before and after the succession of altered cycles [15].

In summary, young male Sprague-Dawley (SD) and Fischer 344 (F) rats were kept 8 weeks on a 24-h cycle with either 10 or 14 h of darkness centered at 0100 h. Pineal NAT and pineal and serum MEL rose during the same time interval in 10 and 14 h darkness in F. In SD, serum MEL rise was delayed in longer dark. Failure to advance the MEL rise in earlier darkness suggests that timing of the rise was not set by late light gating and that its entrainment may be influenced by morning light. MEL variables fell 2 h later in the longer (versus the shorter) darkness despite advance of darkness by 2 h. The fall in MEL variables, complete before lights-on, indicates the MEL fall was not set by light gating. These dynamics fit the two-oscillator entrainment model with predominance of the effect of morning light at these dark phase lengths, more extensively described previously by measurement of NAT, and now extended to circulating MEL with use of two additional rat strains.

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